**Virologica Sinica**

**Supplementary Data**

**Establishment and Characterization of a New Cell Culture System for Hepatitis B Virus Replication and Infection**

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Table S1. Primers used for real-time PCR

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| Primers | Sequence (5’-3’) |
| HNF4α-F  | CGAAGGTCAAGCTATGAGGACA |
| HNF4α-R | ATCTGCGATGCTGGCAATCT |
| NTCP-F | AAGGACAAGGTGCCCTATAAAGG |
| NTCP-R | TTGAGGACGATCCCTATGGTG |
| β-actin-F | CACCAACTGGGACGACAT |
| β-actin-R | ACAGCCTGGATAGCAACG |
| Core-F | AATGCCCCTATCTTATCAACACT |
| Core-R | GAGATTGAGATCTTCTGCGACG |
| pgRNA (HBV2270F) | GAGTGTGGATTCGCACTCC |
| pgRNA (HBV2392R) | GAGGCGAGGGAGTTCTTCT |
| cccDNA (ccc-1582F)cccDNA (ccc-2316R) | TGCACTTCGCTTCACCTGACCACCAAATGCCCCT |



Figure. S1. Determination of optimal DOX concentration for induction of Tet-on system. 7404NT-HNF4α cells were transfected with pTRE-Luc plasmid and cultured in different concentrations of DOX. Luciferase Reporter System Detection kit (Promega) was used to analyze luciferase expression 48 hours later. No obvious changes in cell morphology were observed at the concentrations used.



Figure. S2. Transfection efficiency of chemically synthesized siRNA was compared using FAM-labelled control siRNA to transfect HepG2-NTCP, B7 and G8 cells. Cells cultured in 24-well plates (1.5×105 cells per well) were transfected with 40 nmol per well FAM-siRNA and fluorescence at 48 hours was visualized.